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Characterization Analysis of Response of Alcohol Dehydrogenase Gene (*ADH1*) in *Coix lacroyma jobi* L. to Waterlogging Stress

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Abstract: The aim of this study was focused on response of Alcohol Dehydrogenase gene *(ADH1)* in Coix to waterlogging stress. Based on the conserved sequence of Alcphol Dehydrogenase *(ADH1)* gene in maize, rice, and wheat, primers were designed to isolate the *ADH1* product. The full-length sequence of cDNA was firstly cloned by using RACE technology. The acquired gene contains an open reading frame (ORF, DQ455071.2) of 1140 bp and encodes 379 amino acids residues with the molecular weight and theoretical isoelectric of 40.965 and 6.13 KD, respectively. The BlastN/P analysis revealed that the sequence was highly homologous with gramineous plants such as *ADH1* in maize, rice, and wheat. Moreover, it could be found in the phylogenetic tree that the origin of *ADH1*-encoding protein was most close to gramineous plants. It was predicted that it had at least two standard transmembrane segments, and the three-dimensional structure had 54.38% consistency with the reference model of 2 fzwa. The characteristic belt of *ADH1* target protein was obtained by prokaryotic expression. Semi-quantitative analysis suggested that *ADH1* gene expression was induced by waterlogging, and the expression in the root tip reached the highest level after 4 h of waterlogging, while ADH enzyme activity was also increased after waterlogging and reached the highest level after 6 h. Significant difference occurred in the ADH enzyme activities at different treatment time ($p<0.05$). Results indicated that ADH1 was sensitive to waterlogging and took part in tolerant and adaptive process under anaerobic environment.

Keywords: Alcphol Dehydrogenase (*ADH1*), *Coix lacroyma jobi* L., expression analysis, gene cloning

INTRODUCTION

Waterlogging stress caused by heavy rainfall or poor drainage can cause serious damage to many higher plants due to their aerobic requirements. Of agroeconomic importance is the fact that the majority such plants are important food crops (Dasa *et al*., 2009; Perata and Voesenek, 2007; Drew, 1997). To deal with the negative impacts of complete or partial flooding (Kennedy and Rumpho, 1992; Perata and Alpi, 1993; Fukao *et al*., 2003), plants have evolved effective metabolic adaptation mechanisms to avoid hypoxic stress by developing canopy extensions, aerenchyma development and adventitious root formation. However, such adaptation strategies often lead to a reduction of energy reserves and excessive consumption of carbohydrates (Voesenek *et al*., 2006, 2004). To avoid depleting energy and carbohydrate storage, some plants enter a quiescence mode, which inhibits its growth in hypoxic or anaerobic environments (Bailey-serres and Voesenek, 2008; Colmer and Voesenek, 2009). These observations tend to favor the notion that plants seek a complex, fine balanced mechanism to regulate the processes in volved in the survival adaptation to flooding stress (Fukao and Bailey-serres, 2004; Bailey-serres *et al*., 2010). Recently, the Submergence tolerance (SUB1) gene and ethylene response factor genes, Snorkel1 (SK1) and Snorkel2 (SK2); have been successfully cloned from rain fed low land rice and deep-water rice, respectively (include ref). These are considered to be breakthroughs in the understanding of the mechanism of plant submergence tolerance (Xu *et al*., 2006; Hattori *et al*., 2009; Voesenek and Bailey-Serres, 2008; Hattori *et al*., 2010).

Waterlogging-tolerant plants undergo high levels of ethanol fermentation. This type of metabolism is especially adapted to low oxygen stress by virtue of the active reaction of alcohol dehydrogenase (Liao and Lin, 2001; Dennis *et al*., 2000). It is reported that at least four kinds of *ADH* genes exist in the plants (*ADH1-ADH4*). Mutant which lack the gene for alcohol dehydrogenase is especially sensitive to Waterlogging, suggesting that the fermentation pathway plays an important role in low oxygen tolerance mechanisms (Sauter, 2000; Ellis *et al*., 1999). In addition to waterlogging sensitivity, the *ADH* gene product is also involved in the biotic stress such as chilling and dehydration (Urao *et al*., 1993; Zhu *et al*., 1997). Due

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to the correlation between the level of alcohol dehydrogenase expression and environmental stress tolerance in plants, the *ADH1* genes have been cloned from the important gramineous crops such as *Zea mays*, *Oryza sativa* and the model plant, *Arabidopsis thaliana*, and have been analyzed, respectively (Gerlach *et al*., 1982; Chang and Meyerowitz, 1986; Yong and Ray, 1989).

Coix lacryma jobi L., as a C_4 plant, belongs to gramineae and originates from the tropical and subtropical regions in Southeast Asia. *Coix lacryma jobi* L. is an archaic crop, and is valuable for both it's the main objective of the study was to showed similarities to hygrophyte, which suggests that there may be genes responsible for the waterlogging tolerance phenotype in its genome (Yang, 2007; Ding and Zhang, 1981).

The main objective of the study was to isolate full length of alcohol dehydrogenase gene and analyze its function, and also reveal the molecular mechanism of resistance to waterlogging ecological adaptation in coix. By doing so; we hope to extrapolate our findings to other types of important crops such as *Zea mays* and *Oryza sativa*.

MATERIALS AND METHODS

Plant material: The study was carried out at the Plant development molecular biology laboratory of Yangtze University from September, 2005 till June, 2007. Uniform plumping seeds of *Coix lacryma jobi* L. were selected, and soaked in 0.2 M HNO_3 for 24 h. They were washed for 30 min using tap water, and sowed in sterilized vermiculite. Then, they were transferred to an artificial climatic chamber, watered with ½ MS (Murashige and Skoog) nutrient solution and allowed to germinate under controlled fluctuations in temperature (28ºC/16 h-20ºC/8 h). When three leaves sprouted (ca. 21 days), seedlings with similar heights were selected, and subjected to waterlogging using 1/2 MS nutrient solution. The depth of waterlogging reached the first true leaf. At various times during the waterlogging period, root tips were separated from the main plant for measurement and analysis.

Extraction of total RNA: Plant root tips (0.1 g) were sampled after waterlogging for 6 h, flash frozen in liquid nitrogen and pulverized. Trizol (1 mL) was added, and total RNA was extracted according to manufacturer protocols. The extracted total RNA was used for electrophoresis on a 2% agarose gel. Simultaneously, the OD value (260/280 mm) was measured by an ultraviolet spectrophotometer, and RNA quality and integrity were detected.

Primer sequences and the synthesis: Primer for the first chain synthesis: SMART II A Oligo: 5'-AAG CAG TGG TAT CAA CG CAG AGT ACG CG GG-3'; 3'-CDS: 5'-AAG CAG TGG TAT CAA CGC AGA GTA C (T) 30N-1N-3'; 5'-CDS : 5'- (T) 25N-1N-3' (N = A, C, G or T; N-1 = A, G or C).

Race PCR primer: designed using Primer Premier 5.0, and verified by Oligo 6.0 (http://www.bio-soft.net/).

3'-RACE PCR Gene Specific Primer (GSP1): 5'-ACC CTG AGG CTC CCC TGG ATA AAG T-3'.

3'-RACE Nested Gene Specific Primer (NGSP1): 5'-GAC GGC TGG GGT GTT GCT GTG CTG-3'.

5'-RACE PCR Gene Specific Primer (GSP2): 5'-GGT CAC CAG GGG CAA GGT CAG TCA C-3'.

Universal Primer for RACE PCR (UPM): long fragment: 5'-CTA ATA CGA CTC ACT ATA GGG CAA GCA, GTG GTA TCA ACG CAG AGT-3'; short fragment: 5'-CTA ATA CGA CTC ACT ATA GGG C-3'.

Universal Primer for RACE nested PCR (NUP): 5'-AAG CAG TGG TAT CAA CGC AGA GT-3'.

Primer for Full length cloning of *ADH1*: P1: ATG GCG ACC GCG GGG AAG GT; P2: CTA GTT CTC CAT GCG GAT GAT.

Primer for prokaryotic expression of *ADH1*: P3: CGCGGATCCATGGCGACCGCGGGGAAGGT (containing restriction site of BamH I); P4: CCGCT CGAGCTAGTTCTCCATGCGGATGAT (containing restriction site of Xho I).

All the primers were synthesized in Beijing Augct biotechnological limited company.

***ADH1* gene cloning:** The first chain of cDNA was synthesized according to the instruction of SMARTTM RACE cDNA Amplication Kit from Clonetech company (Takara Biomedical Technology (Beijing) Co., Ltd.). With total RNA (1 μg) as template and the reverse transcription primers in the kits, the first chain of 5'- and 3'-RACE ready, root tip cDNA was amplified using reverse transcription enzymes (Power Script). Following the SMARTTM RACE cDNA Amplication Kit instruction and the provided amplification system, the synthesis of terminal end of 3' RACE and 5' RACE was done using Universal Primer A Mix (UPM) and Nested Universal Primer A (NUP). Nest PCR was used for 3'-RACE amplification under the following conditions: 94°C for 5 min, 94°C for 30s, 68°C for 30s, 72°C for 2 min, 30 cycles and 72°C for 10 min. The first-round PCR product was used as template, nest PCR was repeated under the following conditions: 94°C for 5 min, 94°C for 30s, 65°C for 30s, 72°C for 2 min, 30 cycles and 72ºC for 10 min. Degradation PCR was used for the amplification of 5'-RACE, and the condition was 94°C for 5 min, 94°C for 30s, 70°C for 30s (decrease 2°C in each cycle), 72ºC for 2 min, 30 cycles and 72°C for 10 min, then followed by 94°C for 5 min, 94°C for 30s, 65°C for 30s, 72°C for 2 min, 25 cycles and 72°C for 10 min. All above

products were sequenced to confirm sequence identity. Based on these work, the core sequence of the *ADH1* gene (obtained by the authors' lab, DQ455071), cDNA sequences from 3'-RACE and 5'-RACE were spliced in Vector NT I 8.0 to obtain the cDNA full-length sequence. The open reading frame was found using EditSeq, and accordingly, a pair of primers were designed to amplify the full length cDNA with 3'-RACE-Ready cDNA as the template. The reaction condition was 94°C for 5 min, 94°C for 30s, 61°C for 30s, 72°C for 90s, 72°C for 10 min. The product was serially screened, checked and sequenced at the Shanghai biological engineering technological company.

Prokaryotic expression of *ADH1* gene: Using Positive recombinant plasmid (PMD18-T-*ADH1*) as a template, the *ADH1* gene was amplified using the P3 and P4 primers. The recycled DNA amplification product and PET-32a (+) vector were, respectively subjected to double digestion with restriction endonuclease BamH I and Xho I, and recycled. It was legated by T4 DNA ligase at 16ºC overnight, and transformed into *E. coli* (BL21 (DE3) competent cells. Positive transformed plasmid DNA was isolated, and the sequence was determined after PCR and double digestion. The recombinant plasmid from the bacterial inoculums (1%) was inoculated into LB culture medium containing 100 μg/mL Amp, and culture was shaken overnight at 37ºC. Subsequently, bacterial culture was amplified at a ratio of 1: 100. When the culture reached an OD600 of 0.6, IPTG (0.5 mmol/L) was added to initiate induction. The un-induced recombinant plasmid and empty vector PET32a (+) were used as negative control. After 4h, bacterial cells were obtained, lysed in SDS-PAGE (50 uL) buffer by boiling for 5 min, and centrifuged at 14000 rpm for 15 min. The supernatant was used for 12% SDS-PAGE.

***ADH1 gene* expression analysis in *Coix lacryma jobi* L.:** The internal reference primers, 18sRNA, were based on *Zea mays*. The designed primers were P18S1 5' GTAGTCATATGCTTGTCT 3' and P18S2 5'AA TA TACGCTATTGGAGCTGG 3'. The amplification primers for *ADH1* gene were P1 *ADH1* 5' GTTGG AG AGGGTGTGACTGA 3' and P2*ADH1* 5' GCAAGA CCAACGGCTCCTAA 3'. *Coix lacryma jobi* L. was cultured and treated as described in plant material. The treatment time was 0, 2, 4, 6, 8, 10 and 12 h, respectively. Total RNA was extracted for semi-quantitative PCR analysis according the protocol in extraction of total RNA. Twenty nine cycles were used to amplify the target gene of *ADH1*, and 26 cycles was used to amplify 18 srRNA. The PCR product was stained with ethidium bromide (0.5 μg/mL), and visualized by agarose (1.5% w/v) gel electrophoresis. The gel was digitized using GeneTool (http:// www.biosino.org/ pages/ disk-new.htm). The Relative expression Index (RI) was calculated using the equation: RI = gray value of target gene band/gray value of internal reference band.

Measurement of alcohol dehydrogenase activity: *Coix lacryma jobi* L. seedlings cultured as in the protocol of 2.1 were subjected to waterlogging treatment according to protocol prokaryotic expression of *ADH1* gene. The root tips were processed and the alcohol dehydrogenase activity was measured. Root tip (0.4 g) was first placed in a mortar, combined with 1.6 mL pre-chilled extraction buffer, ground in an ice bath, and centrifuged at 12000 (4ºC) ×g for 20 min. The collected supernatant from this procedure yielded a crude enzyme solution. Alcohol dehydrogenase activity was measured according to the protocol of David *et al*. (1994). The assay was conducted by creating a reaction solution which included 940 μL of 50 mM NAD^+-containing TES buffer (pH7.5; 0.17 mM NAD^+), 50 μL of enzyme extract, and 10 μL of alcohol (40%) to initiate the reaction. The absorbance change was detected at 340 nm, and a 0.01 change of OD value was taken as an enzyme activity Unit (U). The enzyme protein content was determined using the Coomassie Brilliant Blue method. Enzyme activity was calculated as U/mg protein. DPS software (http://www. China dps.net/download.htm) was used for data analysis, and variance analysis was carried out using LSD method.

Analysis of bioinformatics: The nucleotide and amino acid sequences in other species for analysis were both from the NCBI database. The homogenous analysis of nucleotide and amino acid were performed using BlastN/P (http://blast.ncbi.nlm.nih.gov/) in NCBI. *ADH1*cDNA sequence was spliced by Vector NT suit 8.0. Amino acid sequence was translated online by EMBL (http://www.ebi.ac.uk/Tools/st). Multiple comparison of amino acid was finished by ClustalX software. ProtParam was selected to analyze physical and biochemical property. ProtScale was selected to analyze protein hydrophobicity and hydrophilicity (Ex PASy, http://cn.expasy.org). Transmembrane domain-prediction was done in http://www.ch. embnet.org, and verified by TMHMMS erver (http://www.cbs.dtu.dk). Protein secondary structure prediction was operated using the SOPMA web site. Protein three-dimensional structure prediction was done in SWISS-MODEL database. Phylogenetic tree was constructed according to neighbor-joining method using ClustalX and MEGA5.10.

RESULTS AND DISCUSSION

Cloning of *ADH1* gene: Total RNA was extracted for RACE cloning of *ADH1* gene. First, a sequence about 800 bp was amplified by 3' RACE, and subsequently, a

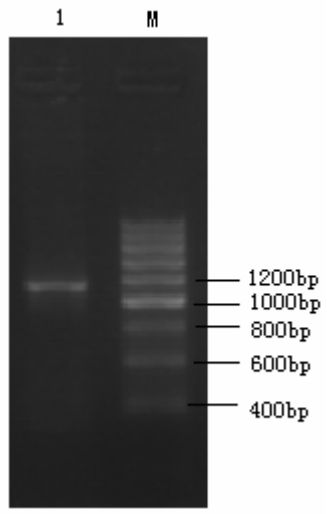

Fig. 1: Electrophoresis profiles of Coix *ADH1* full length on 1.2% agarose gel stained by ethidium bromide (0.5 μg/mL)
Lane l: Coix *ADH1*; M: DNA molecular marker

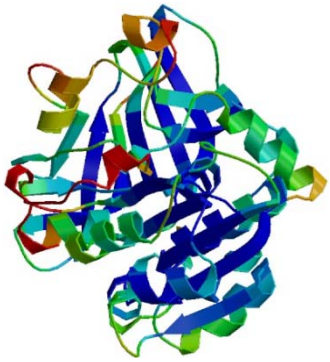

Fig. 2: The prediction of ADH1 tertiary structure in Coix

Table 1: Blast analysis of *ADH1* in *Coix* and other gramineae species

Accession	Species	Query coverage (%)	E value	Max ID (%)
DQ455071.2	*Coix lacryma-jobi*	100	0.0	100
X16547.1	*Pearl millet*	100	0.0	92
HM062764.1	*Miscanthus sinensis*	100	0.0	92
AJ311048.1	*Pennisetum glaucum*	100	0.0	92
NM_001111939.1	*Zea mays*	100	0.0	91
GQ848036.1	*Oryzasativa japonica*	99	0.0	91
AY917130.2	*Phragmites australis*	100	0.0	91
GU798013.1	*Oryzasativa indica*	99	0.0	90
FP092304.1	*Phyllostachys eduli*	100	0.0	90

sequence about 500 bp was amplified by nest PCR. A sequence about 400 bp in length was amplified by 5'RACE using the Touchdown procedure (*ADH1* gene cloning). Primers were next designed according to the spliced sequence, and a specific fragment about 1200 bp was amplified, and the length complied with our prediction (Fig. 1). ORF was obtained through Edit Seq, and was found to be 1140 bp. The initial code was ATG, and the terminal code was TAG. It encoded a protein consisting of 379 amino acids. Accssion: DQ455071 (2007.10): VERSION: DQ455071.2 (2008.12) (http:// www.ncbi.nlm.nih. gov/ nuccore/ DQ455071.2).

Bioinformatics analysis of *ADH1* gene: Blast analysis (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) suggested that the nucleotide sequence of *Coix lacryma jobi* L. *ADH1* had high consistency with the *ADH1* from gramineous plants such as Pearl millet, *Miscanthus sinensis*, *Pennisetum glaucum*, *Oryza sativa*, *Zea mays*, *Phragmites australis* and *Phyllostachys edulis*. The max score was 1000-1600. The query coverage was 90-100%, respectively. The E-value was zero. The max identity reached 90-92% (Table 1).

Simultaneously, homogenous comparison was performed between amino acid sequence of ADH1 in *Coix lacryma jobi* L. and those in other species (http://blast.ncbi.nlm.nih.gov/Blast.cgi#221327759).

The max score was 714-760. The query coverage was 100%. The E-value was zero. The max identity was 96% in *Zea mays*, *Sorghum bicolor*, *Miscanthus sinensis*, *Saccharum*, *Cenchrus americanus* and *Oryza*, while it was 95% in *Triticum aestivum* and 94% in *Aegilops speltoides*, *Brachypodium distachyon* and *Phragmites australis*. In addition, the amino acids of *ADH1* in seventeen plants were selected to compared with the translated amino acid in *Coix lacryma jobi* L. and the result suggested that cDNA full length sequence in *Coix lacryma jobi* L. should be the *ADH1* gene.

Online analysis showed that the molecular weight of protein encoded by *ADH1* was 40965.2 Dalton, and the pI was 6.13. The 251th position of polypeptide chain was Cys, and it had high hydrophilicity. The 202th position of polypeptide chain was Ile, and it had high hydrophobicity. The hydrophilic and hydrophobic amino acids in the entire polypeptide chain were equilibrium distributed. Transmembrane analysis indicated that the protein was located on the inside of cell membrane, and possibly had two potential transmembrane spiral regions which were, respectively between the 172th and 189th amino acid and between 195th and 214th amino acid. Protein secondary structure mainly contained alpha helix, random coil and extended chains. This accounted for 25.59, 38.79 and 25.59%, respectively. Three-dimensional structure was constructed by homology modeling, through positions 4-377 of the amino acid sequence. Lamellar structure constituted the main protein frame. The alpha helix was found to be at the edge of the frame. The basic template for modeling construction was 2 fzw A (1.84 A), and the consistency between them reached 53.48% (Fig. 2).

The ADH1 sequence in some typical species was selected to construct phylogenetic tree together with the sequence derived from *Coix lacryma jobi* L. The result

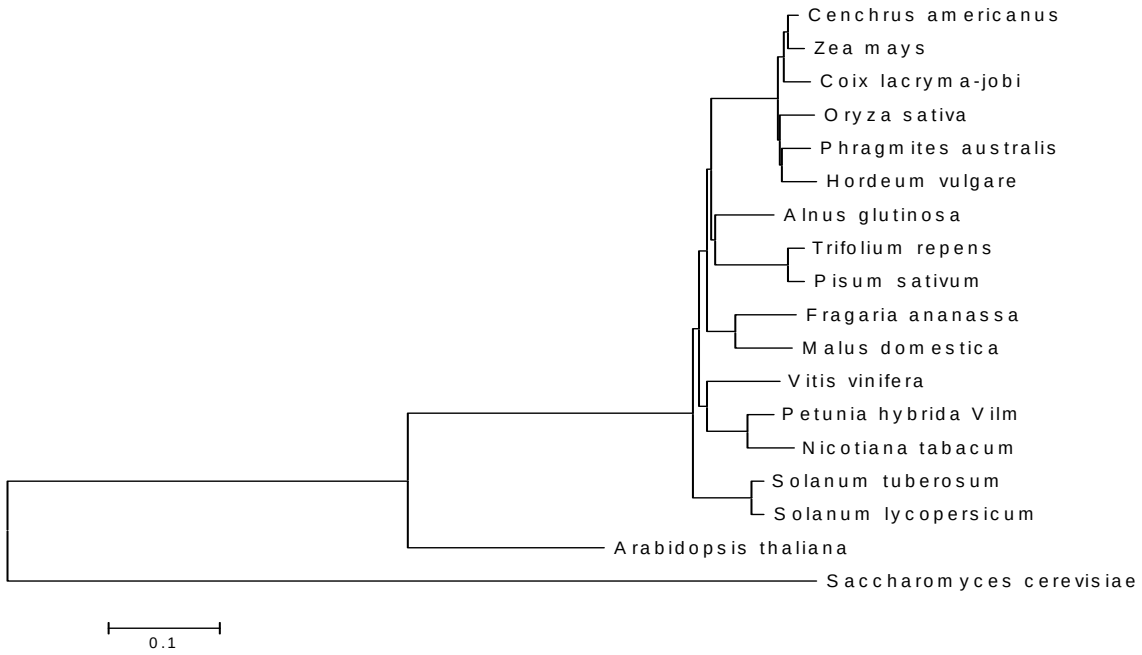

Fig. 3: Phylogenetic tree based on the amino acid of ADH1 in *Coix* and other 17 kinds of species

suggested that *Coix lacryma jobi* L. had most close relationship with *Zea mays* and *Pennisetum glaucum*; Secondly, it had relatively close relationship with *Oryza*, *Phragmites australis* and *Hordeum*; The correlation extent with *Coix lacryma jobi* L. sequentially decreased in the other plants, and the order was *Alnus japonica*>*Trifolium repens*>*Pisum sativum* L.>*Solanum tuberosum* L.>*Solanum lycopersicum*. The most distant relationship was found to be between *Arabidopsis thaliana* and *Coix lacryma jobi* L. and Yeast was located in the most external side, suggesting that no mater yeast or plants, the *ADH1* gene might be evolved from the same ancestor, and they were conservative during the evolvement (Fig. 3).

***ADH1* protein of prokaryotic expression:** PET-32a (+) -*ADH1* was transformed into the BL21 (DE3) cells in E. coli. The cells were induced by IPTG, and used for 12% SDS-PAGE electrophoresis after bacterial protein expression treatment. The detected expression product was 44.5KD and included pET-32a (+) peptide segment (3.5 KD) and *ADH1* (41 KD). The molecular weight was similar to that which was predicted. The control which was not induced by IPTG and the empty vector did not have expression which suggested that this protein sequence would yield to the product of the *ADH1* gene (Fig. 4).

Expression analysis of *ADH1* gene: Root tip (2 μg) was sampled in *Coix lacryma jobi* L. exposed to waterlogging with different time. The RNA was then

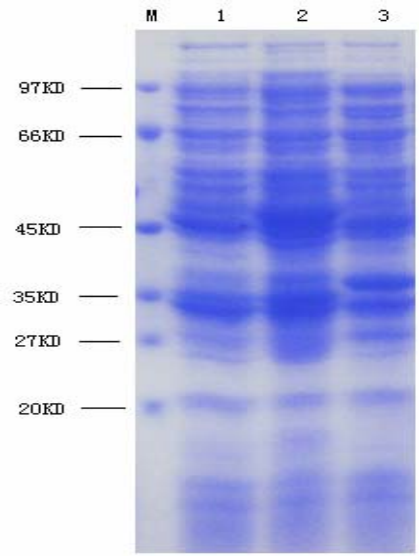

Fig. 4: Electrophoresis profiles of *Coix* ADH1 in prokaryotic expression on SDS-PAGE stained by coomassie brilliant blue

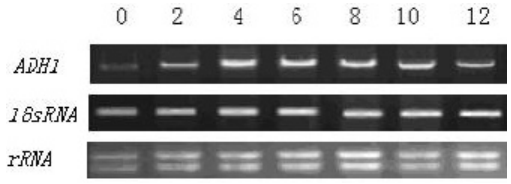

Fig. 5: Electrophoresis profiles of RT-PCR of root apical *ADH1* in response to waterlogging in *Coix*

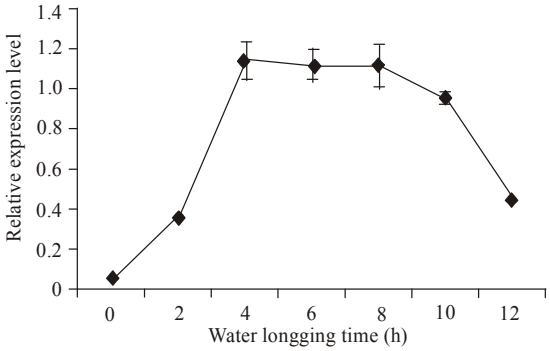

Fig. 6: *ADH1* gene expression patterns of response to waterlogging stress in *Coix* root apical

Table 2: Change in ADH1 enzyme activity with treatment time (T, hour) under waterlogging stress in *Coix* root apical

	Non-waterlogging		waterlogging treatment	
T (h)	ADH activities	Account for CK (%)	ADH activities	Account for CK (%)
0	35.01±1.62b	100	34.51+0.16g	100
2	34.33±0.13bc	98	61.49+0.08f	178
4	32.98±0.14d	94	101.27+0.70e	293
6	34.85±0.05b	99	79.21+0.14a	519
8	36.11±0.98a	103	167.36+0.04b	485
10	33.28±0.15cd	95	157.21+0.13c	456
12	34.38±0.08b	98	130.01+0.12d	377

extracted and reverse transcribed to cDNA. The target and internal reference genes were amplified using cDNA (2 μL) as the template. The PCR product (10 μL) was used for electrophoresis on a 1.5% agarose gel. The result was scanned by gel imaging system (Fig. 5).

As shown in Fig. 5, the root without waterlogging treatment had the lowest *ADH1* expression quantity, and the sequence from gel imaging is very dark. The mRNA level was measurable after 2 h waterlogging, and it reached the highest level after 4 h. From 4 to 8 h, the expression quantity maintained at a steady stage, while it tended to decrease after 10 h. After waterlogging for 12 h, the expression quantity declined significantly. The gray value was analyzed by a GeneTool software. The gray value and the ratio of gray value of internal reference (RI, *ADH1*/18sRNA) were calculated at different time point during waterlogging treatment. The tendency of relative expression quantity following the time was shown in Fig. 6.

***ADH* enzyme activity in roots:** *ADH* enzyme activity was maintained between 33.28 and 36.11 U/mg in the roots without waterlogging treatment. After waterlogging treatment, ADH enzyme activity was higher in the root tip, which indicated a sensitive response to waterlogging. Before waterlogging treatment, ADH enzyme activity was 34.51 U/mg. After waterlogging for 6 h, it increased to 179.21 U/mg, and the increase was 5.2 fold. Until the 12 h, ADH enzyme activity was maintained at 130.01 U/mg. The variance analysis suggested that all the enzyme activity parameters were significantly different at different treatment times ($p<0.05$) (Table 2).

CONCLUSION

Alcohol dehydrogenase genes have previously been cloned from *Zea mays*, *Oryza sativa* and *Arabidopsis thaliana*. However, to date, the full length cDNA of this gene has yet to be cloned from, *Coix lacryma jobi* L. The gene cloned by us is very similar with the cDNA sequence of *ADH1* gene isolated from *Zea mays*. The entire sequence contains the largest ORF (1140 bp), encoding a mature polypeptide of 379 amino acids. Its molecular weight was found to be 40.965 KD, and the isoelectric point was 6.13. It has two important tranmembrane regions between the 172th and 189th amino acid and between 195th and 214th amino acid. Homogenous comparison analysis proved that this sequence was the *ADH1* gene. This gene had high levels of similarity with the *ADH1* in gramineous plants. Phylogenetic tree suggested that no matter yeast or plant, *ADH1* genes all evolved from the same ancestor and was conserved during evolution.

Waterlogging is the main reason for the hypoxia in plant roots (Davletova *et al*., 2005). It was reported that under the hypoxic conditions, *ADH1* mRNA expression quantity in root tip of *Zea mays* rapidly increased in a short period, and reached the highest value at the 6 h. However, the mRNA quantity gradually, decreased with the prolonged anaerobic time and it nearly became zero after 12 h (Kwast *et al*., 1999). Our studyon the responses of *Coix lacryma jobi* L. to waterlogging suggested that after different kinds of waterlogging treatment, the expression quantity of *ADH1* of root tip in *Coix lacryma jobi* L. changed greatly. The expression quantity of was lowest in the un-treated material. In the treated samples, *ADH1* was found to begin accumulating after 2 h of waterlogging and reached the highest value after 6 h treatment, and then decreased, gradually. The decrease was significant after waterlogging treatment for 8 h, and the content was the lowest after 12 h. The *ADH1*expression activity in root tip was higher in *Coix lacryma jobi* L. compared with that in *Zea mays*, and it was also more sensitive to hypoxia, as its mRNA quantity reached a peak value after 4 h, which was 19 fold greater than the un-treated material. *Coix lacryma jobi* L. was similar with *Zea mays* in the time course study of waterlogging stress and *ADH1* gene expression tendency. In the period before waterlogging, *ADH1* expression quantity rapidly increased, but decreased after a period of waterlogging. The possible reason has been illustrated in previous work (Klok *et al*., 2002; Kumar *et al*., 2004; Fliegmann, 1985).

The *ADH1* expression induced by waterlogging associated with the activity of alcohol dehydrogenase.

The result suggested that ADH activity was obviously increased in the root tip of *Coix lacryma jobi* L. exposed to waterlogging compared to that under the normal condition. However, the ADH enzyme activity was not correlated with the waterlogging-induced mRNA quantity of *ADH1*. Expression quantity of *ADH1* gradually decreased from the 4 h onwards, but the increase in enzyme activity continued to the 6 h. Sheila *et al*. pointed out that gene selective translation induced by anaerobic condition was an important regulation mechanism after transcription (Johnson *et al*., 1994). The expression regulation of anaerobic proteins such as ADH not only has transcriptional regulation but also translational regulation (Kelley, 1989; Ismond *et al*., 2003). In addition, the promoter sequence of *ADH1* in evolves response elements to Anaerobic condition (ARE) and specific combined expression of transcriptional factors (AtMYB2) (Olive *et al*., 1990). Translation is a process consuming great energy, and excessive consumption of energy crisis is very high in anaerobic cells. The reduction of synthesis of high energy phosphate bond and decrease of pH in the cytoplasm have previously led to the inhibition of translational initiation, elongation and termination, which possibly affected mRNA expression and its relation with enzyme activity (David *et al*., 1994). On the other hand, our study confirmed again that proper *ADH1* gene expression was necessary for the plant survival under low oxygen conditions. Once the *ADH1* mutation occurred, the anaerobic tolerance in plant was greatly decreased (Kennedy and Rumpho, 1992). It can be proved by the non effective *ADH1* mutants of *Zea mays* and *Arabidopsis thaliana* and *rda* mutant of *Oryza sativa* (low activity of *ADH1*) (Kennedy and Rumpho, 1992; Ellis *et al*., 1999).

Important progress has been obtained in the studies on anaerobic stress in the model plants such as *Oryza sativa* and *Arabidopsis thaliana* (Xu *et al*., 2006; Hattori *et al*., 2009). Taking the cloning of the genes related with survival, tolerance and escape under waterlogging in *Oryza sativa* as the breakthrough, a primary molecular web model has been set up, and it has been concentrated on the N-end regulation and pathway related to response factors of ethylene (Steffens and Sauter, 2010; Gibbs *et al*., 2011; Lee *et al*., 2008; Bailey-Serres *et al*., 2012; Graciet and Wellmer, 2010). It has been revealed that response factors of ethylene had certain relation with *ADH1* gene expression and activity (Peng *et al*., 2001; Licausi *et al*., 2010; Hinz *et al*., 2010; Licausi, 2011). Therefore, based on the cloning of *ADH1* gene in *Coix lacryma jobi* L., the connection between *ADH1* gene expression and response factors (i.e., ethylene) will be further explored by transgenic methods. In all, the data described here may help build a new perspective for plant molecular breeding and improvement.

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